

The impact of temperature on hatching rates of diapause eggs and subsequent development in a tropical freshwater copepod, *Mongolodiptomus malaindosinensis* (Copepoda: Calanoida)

P. Boonmak¹, L. Sanoamuang^{2,3*}

¹ Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et 45120, Thailand.

² Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

³ International College, Khon Kaen University, Khon Kaen 40002, Thailand.

* Corresponding author

Phuttaphanee Boonmak: phuttaphanee@gmail.com ORCID 0000-0003-4827-5532

Laorsri Sanoamuang: la_orsri@kku.ac.th ORCID 0000-0003-0377-1225

ABSTRACT: The diapause egg is an essential component of aquatic invertebrates and is a specialized type of egg that is highly resistant to harsh environmental conditions. The diapause eggs of a tropical freshwater copepod, *Mongolodiptomus malaindosinensis* (Lai et Fernando, 1978), an endemic species of Southeast Asia, were collected from Kaeng Nam Ton Swamp in Khon Kaen province, Thailand. The eggs were isolated by the sugar flotation method and maintained in laboratory conditions at three different temperature levels (25, 30, and 35 °C in an incubator). The first copepod hatching occurred between 6 and 9 days. The average hatching rate per day and the total percentage of eggs hatched in *M. malaindosinensis* were significantly higher ($P < 0.05$) at 25 °C ($9.36 \pm 6.7\%$ and $55.37 \pm 3.1\%$) and significantly lower ($P < 0.05$) at 35 °C ($5.28 \pm 3.5\%$ and $16.67 \pm 2.9\%$), respectively. The post-diapause developmental characteristics of *M. malaindosinensis* have four stages: early development embryo, intermediate development embryo, pre-nauplius, and nauplius. The results suggest that temperature levels affected hatching success in tropical copepods. There is a possibility that the reduced hatching implies that increasing temperatures over a certain threshold may be a limiting factor for its hatching and growth.

How to cite this article: Boonmak P., Sanoamuang L. 2024. The impact of temperature on hatching rates of diapause eggs and subsequent development in a tropical freshwater copepod, *Mongolodiptomus malaindosinensis* (Copepoda: Calanoida) // Invert. Zool. Vol.21. No.3. P.359–368. doi: 10.15298/invertzool.21.3.08

KEY WORDS: crustacean, dormant stage, fisheries, nauplius, Southeast Asia, zooplankton.

Влияние температуры на вылупление из покоящихся яиц и последующее развитие у тропического пресноводного рачка *Mongolodiptomus malaindosinensis* (Copepoda: Calanoida)

П. Бунмак¹, Л. Саноамуанг^{2,3*}

¹ Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et 45120, Thailand.

² Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

³ *International College, Khon Kaen University, Khon Kaen 40002, Thailand.*

* Автор для корреспонденции: la_orsri@kku.ac.th

РЕЗЮМЕ: Диапауза на стадии яйца крайне важна для водных беспозвоночных, так как покоящиеся яйца способны выдерживать неблагоприятные условия среды. Покоящиеся яйца тропического пресноводного веслоногого рака *Mongolodiptomus malaindosinensis* (Lai et Fernando, 1978), эндемика Юго-Восточной Азии, были собраны в болоте Кенг Нам Тон провинции Кхон Кен Таиланда. Яйца были выделены из донных отложений методом флотации в растворе сахара, они инкубировались в лабораторных условиях при трех стабильных температурных режимах (25, 30 и 35 °C). Первые личинки вылуплялись из яиц на 6–9-й день инкубации. Средняя суточная доля развившихся яиц и общее количество развившихся яиц *M. malaindosinensis* были достоверно выше ($P < 0,05$) при температуре 25 °C ($9,36 \pm 6,7\%$ и $55,37 \pm 3,1\%$ соответственно) и достоверно ниже ($P < 0,05$) при температуре 35 °C ($5,28 \pm 3,5\%$ и $16,67 \pm 2,9\%$ соответственно). В развитии *M. malaindosinensis* после диапаузы имеются четыре стадии: ранняя эмбриональная стадия, промежуточная эмбриональная стадия, пре-науплиус и науплиус. Полученные результаты показывают, что уровень температуры может существенно влиять на успех вылупления из покоящихся яиц у тропических копепод. Снижение доли развившихся яиц при повышенных температурах позволяет предположить, что повышение температуры выше определенного уровня может оказаться лимитирующим фактором при развитии копепод из покоящихся яиц.

Как цитировать эту статью: Boonmak P., Sanoamuang L. 2024. The impact of temperature on hatching rates of diapause eggs and subsequent development in a tropical freshwater copepod, *Mongolodiptomus malaindosinensis* (Copepoda: Calanoida) // *Invert. Zool.* Vol.21. No.3. P.359–368. doi: 10.15298/invertzool.21.3.08

КЛЮЧЕВЫЕ СЛОВА: ракообразные, покоящиеся стадии, рыболовство, науплиус, Юго-Восточная Азия, зоопланктон.

Introduction

Copepods play a key role in aquatic ecosystems that provide considerable utility throughout various domains, including fisheries, where cultivated copepods can serve as a vital source of nutrition for fish and shrimp larvae. They are used as natural foods or cultured from natural water bodies (Williamson, Reid, 2001; Boxshall, Defaye, 2008). Copepods provide considerable utility throughout various domains, including fisheries, wherein cultivated copepods can serve as a vital source of nutrition for fish and shrimp larvae. They are used as natural foods and are either cultured or collected from natural water bodies. They have highly unsaturated fatty acids and proteins, which are of great significance for their roles as live feeds, which are essential for the growth and survival of reared larvae in hatcheries (Piasecki *et al.*, 2004; Juntarut, 2014).

Dormant stages provide a mechanism for invertebrates to endure inhospitable environments and to undertake natural long-distance dispersal

(Cáceres, 1997; Arnott *et al.*, 1999; Folt, Burns, 1999). Various invertebrates produce dormant stages such as diapause eggs, resting eggs, or cysts that often sink and accumulate in sediments, forming ‘egg banks’ (Brendonck, De Meester, 2003). Diapause eggs exhibit reduced metabolic activity and disrupted patterns of growth and development (Hutchinson *et al.*, 1999; Zeller *et al.*, 2004). They are highly resistant to harmful environmental conditions, such as food scarcity, drying or freezing of the habitat, and oxygen deficiency, and may persist in habitats that would not permit the survival of regular stages (Alekseev *et al.*, 2023). Diapause eggs are a specialized type of egg produced by some organisms, including calanoid copepods, in response to environmental cues such as changes in temperature, photoperiod (day length), and food availability. It is a period of suspended development or reduced metabolic activity that helps copepods survive unfavorable conditions, such as harsh environmental factors or low food availability (Baumgartner, Tarrant, 2017; Record *et al.*, 2018; Hansen, 2019). Dia-

pause eggs are different from regular eggs in that they have adaptations that enable them to survive adverse conditions for extended periods of time. These adaptations may include thicker and more resilient eggshells or protective coatings. The copepod embryos inside these eggs halt their development and metabolic activities until the conditions become more favorable for growth and survival (Hairston, Van Brunt, 1994; Hairston *et al.*, 1995).

Diapause eggs are an essential adaptive strategy that allows calanoid copepods to cope with unpredictable environmental changes and ensure their long-term survival. This phenomenon has been extensively studied in various species of copepods and contributes to our understanding of the ecological dynamics of aquatic ecosystems (Grice, Marcus, 1981; Dahms, 1995; Sichlau *et al.*, 2011). Embryonic diapause is divided into two types, which are induced by physiological conditions and by a season of the year. Some freshwater copepods, such as *Onychodiptomus sanguineus* (Forbes, 1876), avoid periods of intense fish predation by producing diapausing eggs 1.3 generations before the major onset of fish-induced mortality (Hairston, Munns, 1984). Many populations of marine Calanoida in temperate waters vary in abundance on a seasonal basis, and in coastal areas, the production of resting eggs is common. Embryonic diapause within the egg envelope is common among the Centropagoidea, whether freshwater or marine. In contrast to freshwater species, many marine Centropagoidea produce diapause eggs that are morphologically distinguishable from the regular ones, such as *Paracartia latisetosa* (Krichagin, 1873), a seasonally dominant copepod species in Mediterranean coastal waters. This species is well known for the production of diapause eggs, which allow it to overwinter (Belmonte, Pati, 2007).

Temperature is an important influencing parameter in egg production (Ban *et al.*, 2000; Bonnet *et al.*, 2009; Begum *et al.*, 2012; Liu *et al.*, 2015), embryonic development time (Ban, 1994; Liu *et al.*, 2014), and hatching success (Benni *et al.*, 2010; Tordesillas *et al.*, 2016). The egg production of copepods depends on clutch size and the rate of clutch production and is also related to the cube of the body length. Both of these variables are potentially complex functions of environmental and physiological

conditions (Hopkins, 1977; Watras, 1983). Copepod diapause eggs evolved to synchronize hatching with optimal environmental conditions for larval survival and growth. As temperatures rise, diapause eggs receive cues that indicate suitable conditions for hatching. This temperature-dependent response ensures that copepod larvae emerge when food availability is higher and environmental stressors are minimized (Ban, 1994; Beyrend-Dur, 2010).

A tropical freshwater calanoid copepod, *Mongolodiptomus malaindosinensis* (Lai et Fernando, 1978), is an endemic species of Southeast Asia and is one of the most common copepods in northeast Thailand. It can be found in many lakes, reservoirs, and ponds (Sanoamuang, Dabseepai, 2021; Boonmak, Sanoamuang, 2022; Watroyram, Sanoamuang, 2017). This species has to date been found also in Malaysia (Lai, Fernando, 1978a), Singapore (Lai, Fernando, 1978b), Cambodia (Chaicharoen, Sanoamuang, 2022), and Vietnam (Boonmak, Sanoamuang, 2022). According to our previous study, the life history traits of *M. malaindosinensis* simply depended on food quantity (Boonmak *et al.*, 2018). The main purpose of this study was to determine the responses of the hatching success and development characteristics of diapause eggs of *M. malaindosinensis* tested under different temperature conditions in order to clarify how this tropical copepod responds to temperature changes in the laboratory.

Materials and methods

SEDIMENT COLLECTIONS. Nine samples of sediments were collected by a hand net with a 30 μm mesh size from the littoral zone (depth: 2 m) of three stations located in the north of Kaeng Nam Ton Swamp (16°24'29.97"N, 102°45'39.08"E, altitude 151–153 m), Khon Kaen province, Thailand, on November 28, 2021. The environmental conditions of sampling sites were measured using a PCD650 EUTECH multi-parameter, as follows: Water temperatures ranged from 28.8 to 31.4 °C, pH ranged from 7.62 to 7.85, conductivity ranged from 342 to 420 $\mu\text{S cm}^{-1}$, and dissolved oxygen ranged from 4.1 to 4.8 mg L^{-1} . In the original habitats of *M. malaindosinensis*, water temperatures throughout the year ranged from 25 to 30 °C (Boonmak *et al.*, 2018). Then, the sediments were spooned into a 180-mL plastic bottle containing a small amount of natural water and placed in an incubator (MLR-350, Sanyo, Japan) at 25 °C, and they were stored in the labora-

Table 1. The mean values and standard deviations (SD) of the hatching rate (HR) per day and the total percentage of eggs hatched in *Mongolodiptomus malaindosinensis* (n , number of replicates in each treatment).

Таблица 1. Средние значения и стандартное отклонение (SD) ежедневной доли развившихся яиц (HR) и общий процент развившихся яиц у *Mongolodiptomus malaindosinensis* (n , количество повторностей в каждой серии).

Hatching rate (%)	Temperature (°C)								
	25			30			35		
	Mean	SD	n	Mean	SD	N	Mean	SD	n
HR per day	9.36 ^a	6.70	3	7.22 ^a	3.47	3	5.28 ^b	3.51	3
Total HR	55.37 ^a	3.06	3	31.48 ^b	1.16	3	16.67 ^c	2.91	3

Values with the same superscript characters in each test indicate no significant difference among temperatures (post-hoc Duncan test, $df = 2$, $P < 0.05$ for both).

tory for 120 days before being used in the hatching experiments.

EXPERIMENTAL CONDITIONS. Diapause eggs of calanoid copepods were separated from the sediments by the sugar flotation method (Ban, Minoda, 1992). The unsterilized sediments were sieved through a 100- μm mesh and then a 30- μm mesh screen. After that, the sediments on the 30- μm mesh screen were placed in a centrifuge tube containing a dense sugar solution (1 kg L⁻¹). The solution was stirred and then centrifuged at 3,000 rpm for 5 minutes. The supernatant was immediately placed in a 10- μm mesh sieve and rinsed with distilled water to remove the sugar. The material remaining on the 30- μm mesh sieve was washed with filtered (Whatman GF/C) tap water into a Petri dish. Diapause eggs of calanoid copepods were counted under a compound microscope and transferred with a fine glass capillary tube from the dish to another one containing filtered tap water.

The average water temperature in freshwater resources in northeast Thailand throughout the year ranged from 25 to 30 °C (Department of Irrigation, 2022). In Kaeng Nam Ton Swamp, the animals experience water temperatures ranging between 26 and 31 °C (Phugonkchana *et al.*, 2017). The experimental temperatures were therefore selected at three different temperatures (25, 30, and 35 °C), providing a range between the lowest and highest temperatures in Kaeng Nam Ton Swamp. At each temperature, 150 eggs were used with three replicates. Thus, a total of 1350 eggs were placed individually in 5-mL 48-well polystyrene tissue-culture plates (TR5000; Trueline, Romeoville, Illinois, U.S.A.). Eggs were filled with filtered tap water and maintained at the three temperatures in an incubator (MIR-254, Panasonic). For each temperature, the hatching rate of the eggs was checked twice per day under a dissecting microscope (Olympus, SZX12, Japan) at ca. 10–40 \times magnification. The observed time in 30 days was considered the endpoint. The egg hatching rate (%) for each replicate well was then calculated as a percentage in

relation to the initial number of eggs introduced in each replicate well. The post-diapause development characteristic of *M. malaindosinensis* was checked at 40 \times magnification under a light microscope (Optika B-1000FL-HBO, Italy) with a camera lens exfocus at 0.66 \times magnification (Optika, Italy).

STATISTICAL ANALYSIS. A one-way ANOVA was used to determine the differences in the effect of various temperatures on egg hatching success. Standardized data were explored to detect outliers, then $\log_{10}[x + 1]$ was transformed before performing statistical analyses to decrease the variance of the data set and to avoid violating assumptions of normality. All statistical analyses were performed with SPSS 19.0 software (IBM Inc., 2011). The significance level was set at $P < 0.05$.

Results

HATCHING SUCCESS OF DIAPAUSE EGGS. The first embryos of *M. malaindosinensis* emerged from the diapause eggs on day 6 at 30 °C, followed by days 7 and 9 at 25 and 35 °C, respectively. The total percentage of eggs hatched varied significantly ($df = 2$, $F = 101.653$, $P < 0.05$) at different temperatures and was highest at 25 °C (averages: $55.37 \pm 3.1\%$) and lowest at 35 °C (averages: $16.67 \pm 2.9\%$) (Table 1).

Egg hatching rates per day varied significantly ($df = 2$, $F = 4.987$, $P < 0.05$) at different temperatures, and the lowest hatching rates averaged $5.28 \pm 3.5\%$ at 35 °C. At other temperatures, the mean hatching rates varied between $9.36 \pm 6.7\%$ and $7.22 \pm 3.5\%$ at 25 and 30 °C, respectively (Table 1). The egg incubation period at 25 °C was between 7 and 14 days, and at other temperatures, the range of egg incubation periods varied between 6 and 11 and 9 and 12 days at

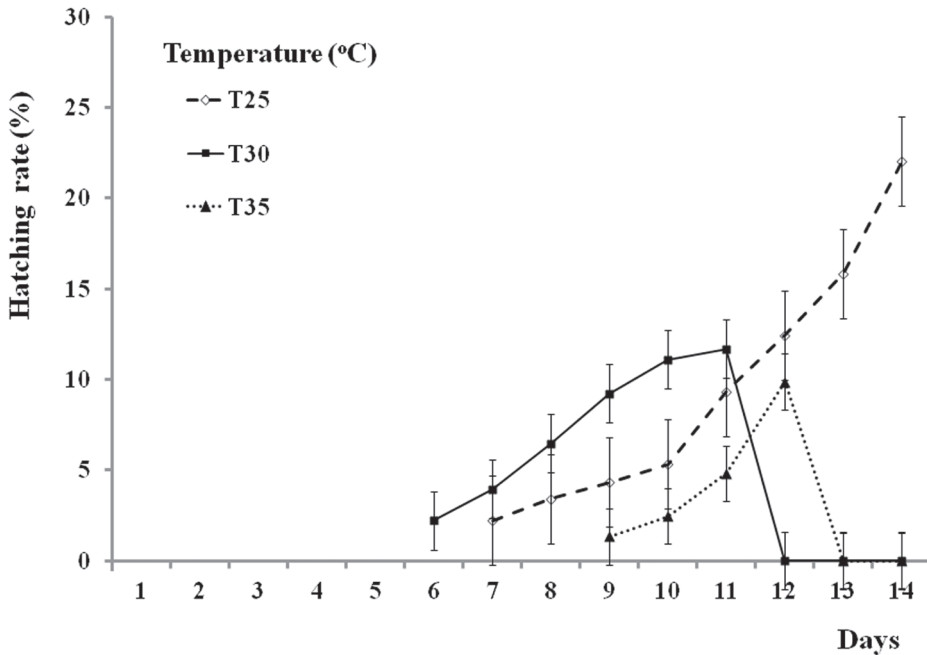


Fig. 1. The hatching percentages of diapause eggs of *Mongolodiptomus malaindosinensis* in cultures for 14 days at three different temperatures.

Рис. 1. Процент развившихся покоящихся яиц *Mongolodiptomus malaindosinensis* в культуре за 14 дней при трех разных температурах.

30 and 35 °C, respectively (Fig. 1). One-way ANOVA demonstrated that there were significant differences among different temperatures on days 8, 9, 10, and 11, but on days 7 and 12, there were no significant differences in hatching rates at each temperature. Statistically, a linear relationship between the temperature and the hatching rate was evident during the study; the values are expressed in the following equation: Hatching rate = $-3.87x + 150.61$ ($R^2 = 0.982$, $P < 0.05$).

POST-DIAPAUSE DEVELOPMENT. Developmental patterning of the embryo was observed under a light microscope at 40x magnification with a camera lens exfocus at 0.66x magnification (Fig. 2). The majority of embryos of *M. malaindosinensis* were isolated from sediments in a pond (Fig. 1A). These embryos were presumed to be in the diapauses stage because they did not develop, hatch, or deteriorate when isolated from the sediment and incubated at 25 °C under aerobic conditions with constant light or a 12:12 light: dark cycle to mimic conditions of

the natural weather in Thailand. Live embryos in the diapause stage could not be differentiated from embryos in an early stage of post-diapause development with light microscopy, so these were grouped together and designated as the Early Development (ED) embryonic stage (Fig. 1A). Development in progress first became apparent when bilaterally symmetrical spaces appeared between the cyst wall and the inner embryo mass at one end of the embryo in a stage designated as the Intermediate Development (ID) embryonic stage (Fig. 1B–C). There was some variability in the timing of individual developments. Reaching the ID embryonic stage within 24–48 hours, the individual progressed to the pre-nauplius stage, characterized by an oval shape with well-defined bilateral symmetry and body axis formation (Fig. 1D). The jerking of the body was visible late in the pre-nauplius stage. Hatching of the nauplius larva started with the rupture of the outer cyst wall (Fig. 1E) and the complete emergence of the nauplius inside a flexible layer structure that is shed in two stages as the space surrounding the

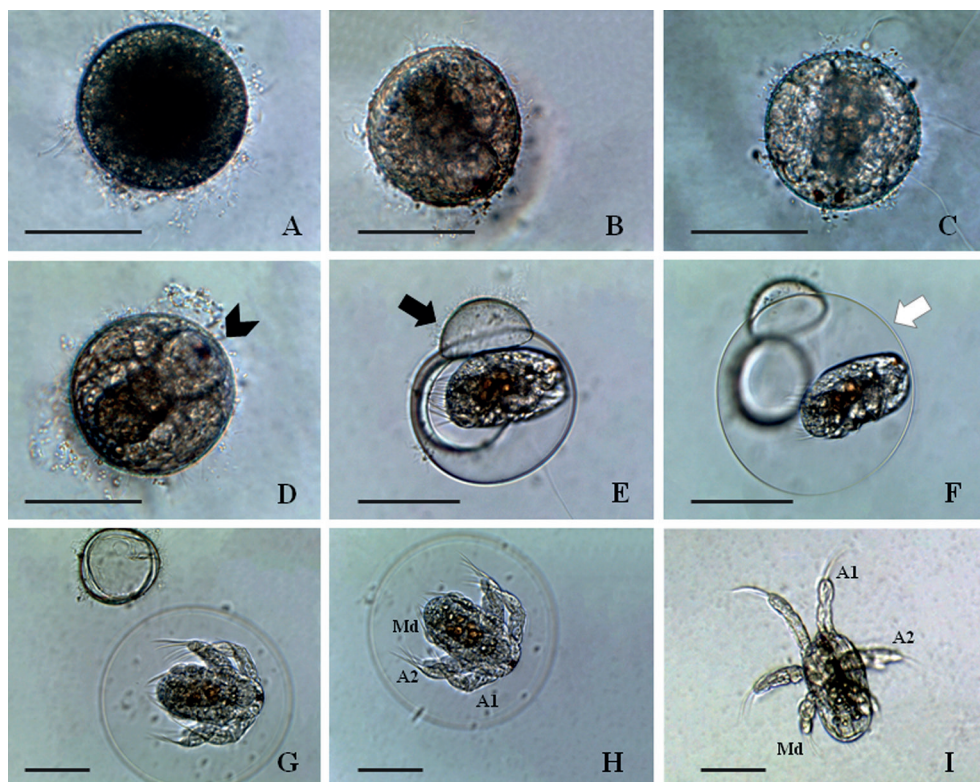


Fig. 2. Morphological characteristics of *Mongolodiptomus malaindosinensis* during post-diapause development, emergence, and hatching. A — early stages of development; B–C — intermediate stages of development; D — pre-nauplius with an ocellus (black arrowhead); E — emergent pre-nauplius and outer wall (black arrow) of cyst shed; F — inner membrane (white arrow); G–H — inner membrane fully expanded; nauplius begins to burst swimming; I — free-swimming nauplius larva. Abbreviations: A1 — antennule; A2 — antenna; Md — mandible. Scale bars — 100 μ m.

Рис. 2. Морфология *Mongolodiptomus malaindosinensis* во время развития из покоящегося яйца и вылупления: А — ранняя стадия эмбрионального развития; В–С — промежуточные стадии эмбрионального развития; D — пре-науплиус с науплиальным глазом (показан стрелкой); E — вылупившийся пре-науплиус внешняя стенка сброшенной цисты (показана стрелкой); F — внутренняя мембрана (показана стрелкой); G–H — внутренняя мембрана полностью расправлена, науплиус начинает спорадические плавательные движения; I — свободно плавающий науплиус. Обозначения: A1 — антеннула; A2 — антенна; Md — мандибула. Масштаб 100 μ m.

nauplius expands and a thin hatching membrane is stretched (Fig. 1F–H). The nauplius rushed until the thin hatching membrane ruptured, releasing the free-swimming nauplius (Fig. 1I).

Discussion

Both the egg hatching rates per day and the total percentage of eggs hatched in *Mongolodiptomus malaindosinensis* were considerably higher at 25 $^{\circ}$ C, in contrast to the increasing temperature at 35 $^{\circ}$ C, where hatching rates were

negatively affected by the highest temperature. The hatching success of eggs in tropical copepods can indeed be affected by high temperatures. Elevated temperatures can have a significant impact on the reproductive and developmental processes of many aquatic organisms. The sudden decline in hatching success at the highest temperature tested can be attributed to severe thermal stress, as has been observed for other calanoid copepods (Lee *et al.*, 2003; Rhyne *et al.*, 2009; Yoshida *et al.*, 2012). Effects of high temperatures can often lead to a decrease in the

percentage of copepod eggs that successfully hatch because copepod embryos are sensitive to temperature changes, and temperatures above their optimal range can disrupt their development (Jiménez-Melero *et al.*, 2005, 2012; Liu *et al.*, 2014, 2015). The significantly high rates of hatching success of diapause eggs in *M. malaindosinensis* at 25 °C suggested that this is the optimal temperature for hatching, and an increase in temperature beyond this range may limit the population growth and reproduction of *M. malaindosinensis* (Boonmak *et al.*, 2018).

While copepods are ectothermic organisms, excessively high temperatures can accelerate their metabolic rates and shorten the time they spend in the egg stage. It can also lead to premature hatching, which reduces the chances of successful survival and recruitment of copepod populations. Moreover, elevated temperatures can induce stress in copepod embryos, affecting their metabolic processes and overall development. This stress can lead to a higher rate of egg mortality and lower hatching success (Patterson *et al.*, 2021; Reed *et al.*, 2021). Many studies have shown that varying temperature regimes can influence the timing and synchronization of diapause egg hatching, impacting the success of copepod generations. Warmer temperatures can accelerate hatching rates, potentially leading to earlier emergence and altered population dynamics (Monchenko, 1996). However, the impact of high temperatures on copepod hatching success is not uniform across all species and populations. Different species and populations have varying temperature tolerances and thresholds. For example, the diapause egg-hatching success of the copepod *Acartia tonsa* Dana, 1849, increased with increasing temperature up to a certain point and then declined, and the optimal temperature range for hatching was between 18 and 22 °C (Kiorboe, Hirst, 2008). For temperate species, the diapause egg hatching of *Calanus glacialis* Jaschnov, 1955 and *C. ponticus* Krichagin, 1873 in the Arctic Ocean found that hatching success was higher at lower temperatures (between 0 and 4 °C) and decreased at higher temperatures; this may be due to the copepods being adapted to the cold Arctic environment (Häfker *et al.*, 2018; Kosobokova *et al.*, 2011).

Diapause eggs of *M. malaindosinensis* used a longer hatching time (day 7 at 25 °C) than regular eggs, which are one to three days (authors'

unpublished data indicate that in a preliminary study, the regular eggs of *M. malaindosinensis* hatching emerged between 24 and 60 hours and the mean hatching success was 85% at 25 °C). Generally, depending on the temperature of the water, copepod embryogenesis can take between 8 and 48 hours, after which a nauplius larva will emerge from the egg (Roman, Pierson, 2022). The lower hatching percentage of copepod diapause eggs compared to regular eggs can be attributed to several factors related to diapause, which is a period of dormancy or suspended development commonly observed in many organisms. Diapause eggs are designed to remain dormant for an extended period, often until environmental conditions become more favorable for the survival and growth of the offspring. That can lead to a delay in hatching, reducing the percentage of diapause eggs that successfully hatch compared to regular eggs (Dussart, Defaye, 2001). Including, diapause eggs may have evolved to resist hatching cues until they have experienced a sufficient duration of the cues simultaneously (Belmonte, Pati, 2007). Moreover, metabolic processes in copepod eggs are typically suppressed to conserve energy (Liu, Ban, 2017). Diapause eggs often require specific environmental cues to trigger hatching, such as changes in temperature, salinity, or light conditions (Epp, Lewis, 1980; Hirche *et al.*, 1997). However, it may be found that the hatching success of diapause eggs of some marine copepods, *Centropages hamatus* (Lilljeborg, 1853), was higher than 80% for eggs stored for 4–17 months at 25 °C (Marcus, Murray, 2001).

Previous studies on *M. malaindosinensis* describe the effects of food variables on life history traits, embryo development time, and survival in live copepods and their regular eggs (Boonmak *et al.*, 2018), but none provide a description of diapause developmental events that can be used to characterize the impacts of environmental variables on development. In the present work, the diapause development of *M. malaindosinensis* was described based on the Antarctic freshwater copepod *Boeckella poppei* (Mrazek, 1901), which fully describes encysted post-diapause development in a member of the genus *Boeckella* in which emergence and hatching depend on the osmotic expansion of a space between a hatching membrane and the fully formed nauplius larva (Reed *et al.*, 2018, 2021). In *M. malaindosinensis*,

the number of membrane layers was not clear, it has been observed that the outer layers matching the dimensions of the diapause embryo are shed sequentially during expansion of this space, and the hatching membrane eventually ruptures as a consequence of burst swimming by the nauplius. This event described previously for *B. poppei* (Burns, 1980; Jamieson, 1988; Hall, Burns, 2001; Reed *et al.*, 2018) and the brine shrimp, *Artemia franciscana* Kellogg, 1906 (Covi *et al.*, 2016) is similar to that of *M. malaindosinensis*, but the proportional expansion of the space between the hatching membrane and nauplius larva is larger in *M. malaindosinensis*. Previous studies in *Artemia* sp. reported that emergence and hatching may depend on the action of a chitinase working in concert with osmotic swelling, but the role of chitinases in the emergence and hatching of copepods remains unexplored (Rosowski *et al.*, 1997). The physiological mechanisms that regulate the transition from diapause to post-diapause in many copepod species are still unknown. At present, transcriptional analysis is used to find hypotheses related to potential mechanisms that terminate diapause before an organism can resume its development (Lenz *et al.*, 2021; Roncalli *et al.*, 2021).

Conclusions

The results of this study indicate that the temperature of 25 °C is suitable for hatching in *Mongolodiptomus malaindosinensis*, as shown by the relatively high rates of hatching success observed. Additionally, it is plausible that elevated temperatures beyond a certain threshold may significantly impact the population dynamics and reproductive capabilities of tropical copepods, as shown by the reduced hatching success observed. This suggests that high temperatures could potentially act as a limiting factor for population growth and reproduction in this species. The effect of temperature on the diapause egg hatching of copepods appears to vary depending on the species and the temperature range examined. Optimal hatching temperatures have been found to range from 2 to 25 °C, with some species showing higher hatching success at lower temperatures. The timing of diapause onset may also play a role in determining hatching success at different temperatures. Overall, further research is needed to better understand the mechanisms

underlying the effect of temperature on diapause egg hatching in copepods.

Funding

This work was funded by a grant from the Thailand Science Research and Innovation (TSRI) in the Fundamental Fund 2022 category.

Author contribution statement

PB was responsible for the literature review, sediment collection, methodology, photographs, preparation of figures, and the first draft of the manuscript. LS was responsible for conceptualization, writing and editing of the final manuscript, and general revisions.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

Acknowledgements. The authors thank Dr. Xin Liu for his technical training and consultation in the sugar flotation method. The authors gratefully acknowledge Russell J. Shiel for his critical review and linguistic corrections to the manuscript. The authors thank Weerathan Maskasem and Prapatsorn Dabseepai for field assistance.

References

- Alekseev V., Dahms H.U., Hwang J.S., Sukhikh N. 2023. Invasive and rare aquatic invertebrates of Taiwan with a focus on their dormancy // Water. Vol.15. Art.3155. <https://doi.org/10.3390/w15173155>
- Arnott S.E., Yan N.D., Magnuson J.J., Frost T.M. 1999. Inter annual variability and species turnover of crustacean zooplankton in Shield lakes // Can. J. Fish. Aquat. Sci. Vol.56. P.162–172.
- Ban S. 1994. Effect of temperature and food concentration on post-embryonic development, egg production and adult body size of calanoid copepod *Eurytemora affinis* // J. Plankton Res. Vol.16. P.721–735.
- Ban S., Minoda T. 1992. Hatching of diapause eggs of *Eurytemora affinis* (Copepoda: Calanoida) from lake bottom sediment // J. Crustac. Biol. Vol.12. No.1. P.51–56.
- Ban S., Lee H.W., Shinada A., Toda T. 2000. *In situ* egg production and hatching success of the marine copepod *Pseudocalanus newmani* in Funka Bay and adjacent waters off southwestern Hokkaido, Japan: associated to diatom bloom // J. Plankton Res. Vol.22. P.907–922.
- Baumgartner M.F., Tarrant A.M. 2017. The physiology and ecology of diapause in marine copepods // Annu. Rev. Mar. Sci. Vol.9. P.387–411.
- Begum B.D., Dharani G., Altuff K. 2012. Effect of temperature on the egg production and hatching success of *Sinodiptomus (Rhinediptomus) indicus* (Calanoida: Copepoda) // Afr. J. Basic. Appl. Sci. Vol.4. No.6. P.216–220.
- Belmonte G., Pati A.C. 2007. Hatching rate and diapause duration in eggs of *Paracartia latisetosa* (Copepoda:

- Calanoida) // J. Plankton Res. Vol.29. Suppl.1. P.39–47. <https://doi.org/10.1093/plankt/fbl064>
- Benni W.H., Drillet G., Kozmér A., Madsen K.V., Pedersen M.F., Sørensen T.F. 2010. Temperature effects on copepod egg hatching: does acclimatization matter // J. Plankton Res. Vol.32. No.3. P.305–315.
- Beyrend-Dur D. 2010. Life history traits of key brackish copepods from temperate to tropical environments. University of Lille, France. 216 p.
- Bonnet D., Harris R.P., Yebra L., Guilhaumon F., Conway D.V.P., Hirst A.G. 2009. Temperature effects on *Calanus helgolandicus* (Copepoda: Calanoida) development time and egg production // J. Plankton Res. Vol.3. No.1. P.31–44.
- Boonmak P., Liu X., Ban S., Sanoamuang L. 2018. Effects of different algal diets and carbon supplies on larval development, growth and survival of *Mongolodiatomus malaindosinensis* (Copepoda: Calanoida) collected from a tropical pond in Thailand // Plankton Benthos Res. Vol.13. No.4. P.163–172. <https://doi.org/10.3800/pbr.13.163>
- Boonmak P., Sanoamuang L. 2022. Diversity of freshwater calanoid copepods (Crustacea: Copepoda: Calanoida) in Southern Vietnam with an updated checklist for the country // Diversity. Vol.14. Art.523. <https://doi.org/10.3390/d14070523>
- Boxshall G.A., Defaye D. 2008. Global diversity of copepods (Crustacea: Copepoda) in freshwater // Hydrobiologia. Vol.595. No.1. P.195–207.
- Brendonck L., De Meester L. 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archive in the sediment // Hydrobiologia. Vol.491. P.65–84.
- Burns C.W. 1980. Instar development rates and production of three generations of *Boeckella dilatata* (Copepoda: Calanoida) in a warm monomictic lake // SIL Proc. Vol.21. P.1578–1583.
- Cáceres C.E. 1997. Dormancy in invertebrates // Invertebr. Biol. Vol.116. P.371–383.
- Chaicharoen R., Sanoamuang L. 2022. Distribution and diversity of diaptomid copepods in freshwater habitats of Cambodia (Crustacea: Copepoda: Calanoida: Diaptomidae) // Diversity. Vol.14. Art.903. <https://doi.org/10.3390/d14110903>
- Covi J.A., Hutchison E.R., Neumeyer C.H., Gunderson M.D. 2016. Rotenone decreases hatching success in brine shrimp embryos by blocking development: implications for zooplankton egg banks // PLoS One. Vol.11. Art.e0163231.
- Dahms H.U. 1995. Dormancy in the Copepoda: an overview // Hydrobiologia Vol.306. P.199–211.
- Department of Irrigation. 2022. Annual water quality report. Ministry of Agriculture and Cooperatives, Thailand. 129 p.
- Dussart B.H., Defaye D. 2001. Introduction to the Copepoda // H.J. Dumont (ed.). Guides to the identification of the microinvertebrates of the continental waters of the world. The Netherlands: SPB Academic Publishing. P.1–344.
- Epp R.W., Lewis W.M. 1980. The nature and ecological significance of metabolic changes during the life history of copepods // Ecology. Vol.61. P.259–264.
- Folt C.L., Burns C.W. 1999. Biological drivers of zooplankton patchiness // Trends Ecol. Evol. Vol.14. P.300–305.
- Grice G.D., Marcus N.H. 1981. Dormant eggs of marine copepods // Oceanogr. Mar. Biol. Annu. Rev. Vol.19. P.125–140.
- Häfker N.S., Teschke M., Last K.S., Pond D.W., Hüppe L., Meyer B. 2018. *Calanus finmarchicus* seasonal cycle and diapause in relation to gene expression, physiology, and endogenous clocks // Limnol. Oceanogr. Vol.63. P.2815–2838. <https://doi.org/10.1002/lno.11011>
- Hairston N.G., Munns W.R. 1984. The timing of copepod diapause as an evolutionarily stable strategy // Am. Nat. Vol.123. No.6. P.733–751. <http://www.jstor.org/stable/2460897>
- Hairston N.G., Van Brunt R.A. 1994. Diapause dynamics of two diaptomid copepod species in a large lake // Hydrobiologia. Vol.292–293. P.209–218.
- Hairston N.G., Van Brunt R.A., Kearns C.M., Ergstrom D.R. 1995. Age and survivorship of diapausing eggs in a sediment egg bank // Ecology. Vol.76. P.1706–1711.
- Hall C.J., Burns C.W. 2001. Hatching of *Boeckella hamata* (Copepoda: Calanoida) resting eggs from sediments of a tidally influenced lake // N.Z. J. Mar. Freshwat. Res. Vol.35. P.235–238.
- Hansen B.W. 2019. Copepod embryonic dormancy: An egg is not just an egg // Biol. Bull. Vol.237. No.2. P.145–169.
- Hirche H., Meyer U., Niehoff B. 1997. Egg production of *Calanus finmarchicus*: effect of temperature, food and season // Mar. Biol. Vol.127. P.609–620.
- Hopkins C. 1977. The relationship between maternal body size and clutch size, development time and egg mortality in *Euchaeta norvegica* (Copepoda: Calanoida) from Loch Etive, Scotland // J. Mar. Biol. Assoc. UK. Vol.57. No.3. P.723–733.
- Hutchinson T.H., Pounds N.A., Hampel M., Williams T.D. 1999. Life-cycle studies with marine copepods (*Tisbe battagliai*) exposed to 20-hydroxyecdysone and diethylstilbestrol // Environ. Toxicol. Chem. Vol.18. No.12. P.2914–2920. <https://doi.org/10.1897/1551-5028>
- IBM Inc. 2011. IBM SPSS Statistics (predictive analytics software and solutions) Version 19.0
- Jamieson C.D. 1988. Variations in the embryonic development times and hatching success of three *Boeckella* species (Copepoda: Calanoida) // Hydrobiologia. Vol.160. P.79–84.
- Jiménez-Melero R., Santer B., Guerrero F. 2005. Embryonic and naupliar development of *Eudiaptomus gracilis* and *Eudiaptomus graciloides* at different temperatures: Comments on individual variability // J. Plankton Res. Vol.27. P.1175–1187.
- Jiménez-Melero R., Parra G., Guerrero F. 2012. Effect of temperature, food and individual variability on the embryonic development time and fecundity of *Arctodiatomus salinus* (Copepoda: Calanoida) from a shallow saline pond // Hydrobiologia. Vol.686. P.241–256.
- Juntarut P. 2014. Roles of copepods in larviculture // KKU Res. J. Vol.19. No.6. P.939–949.
- Kjørboe T., Hirst A.G. 2008. Optimal development time in pelagic copepods // Mar. Ecol. Prog. Ser. Vol.367. P.15–22.
- Kosobokova K., Hopcroft R., Hirche H.J. 2011. Patterns of zooplankton diversity through the depths of the Arctic's central basins // Mar. Biodivers. Vol.41. P.29–50. <https://doi.org/10.1007/s12526-010-0057-9>
- Lai H.C., Fernando C.H. 1978a. Redescription of *Neodiatomus butulifer* Kiefer and one of its related species // Hydrobiologia. Vol.59. P.229–235.
- Lai H.C., Fernando C.H. 1978b. The freshwater Calanoida (Crustacea: Copepoda) of Singapore and peninsular Malaysia // Hydrobiologia. Vol.61. P.113–127.

- Lee H., Ban S., Ikeda T., Matsuishi T. 2003. Effect of temperature on development growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food condition // *J. Plankton Res.* Vol.25. P.261–271.
- Lenz P.H., Roncalli V., Cieslak M.C., Tarrant A.M., Castelfranco A.M., Hartline, D.K. 2021. Diapause vs. reproductive programs: transcriptional phenotypes in a keystone copepod // *Commun. Biol.* Vol.4. Art.426. <https://doi.org/10.1038/s42003-021-01946-0>
- Liu X., Ban S. 2017. Effects of acclimatization on metabolic plasticity of *Eodiaptomus japonicus* (Copepoda: Calanoida) determined using an optical oxygen meter // *J. Plankton Res.* Vol.39. P.111–121.
- Liu X., Beyrend-Dur D., Dur G., Ban S. 2014. Effects of temperature on life history traits of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan) // *Limnology.* Vol.15. P.85–97.
- Liu X., Beyrend-Dur D., Dur G., Ban S. 2015. Combined effects of temperature and food concentration on growth and reproduction of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan) // *Freshwater Biol.* Vol.60. P.2003–2018.
- Marcus N.H., Murray M. 2001. Copepod diapause eggs: A potential source of nauplii for aquaculture // *Aquaculture.* Vol.201. No.1. P.107–115.
- Monchenko V.I. 1996. The problem of induction and termination of diapause in cyclopoid copepods // *Hydrobiologia.* Vol.320. P.119–122. <https://doi.org/10.1007/BF00016812>
- Patterson L.N., Paulson D.M., Colucciello V.J., Covi J.I. 2021. Sediment from lake with missing egg bank is toxic to hatching of model zooplankton: A reason to consider obligate dormancy in toxicological assessment // *Aquat. Toxicol.* Vol.236. Art.105862. <https://doi.org/105862.10.1016/j.aquatox.2021.105862>
- Phugonkchana Y., Wiriyapattanasub P., Wongmaneeprateep S. 2017. The relationship between phytoplankton and some water quality in Bung Kaeng Nam Ton, Muang District, Khon Kaen Province, Thailand // *Khon Kaen Agr. J.* Vol.45. Suppl.1. P.145–151.
- Piasecki W., Goodwin A.E., Eiras J.C., Nowak B.F. 2004. Importance of Copepoda in freshwater aquaculture // *Zool. Stud.* Vol.43. No.2. P.193–205.
- Record N.R., Ji R., Maps F., Varpe O., Runge J.A., Petrik C.M., Johns D. 2018. Copepod diapause and the biogeography of the marine lipidic cape // *J. Biogeogr.* Vol.45. P.2238–2251. <https://doi.org/10.1111/jbi.13414>
- Reed K., Lee S., Lee J., Park H., Covi J. 2021. The ultrastructure of resurrection: Post-diapause development in an Antarctic freshwater copepod // *J. Struct. Biol.* Vol.213. Art.107705. <https://doi.org/10.1016/j.jsb.2021.107705>
- Reed K., Park H., Lee S., Lee W., Lee S., Bleau J., Munden T., Covi J. 2018. Embryos of an Antarctic zooplankton require anoxia for dormancy, are permeable to lipophilic chemicals, and reside in sediments containing PCBs // *Sci. Rep.* Vol.8. <https://doi.org/10.1038/s41598-018-34689-w>
- Rhyne A., Ohs C.L., Stenn E. 2009. Effects of temperature on reproduction and survival of the calanoid copepod *Pseudodiaptomus pelagicus* // *Aquaculture.* Vol.292. No.1. P.53–59.
- Roman M.R., Pierson J.J. 2022. Interactive effects of increasing temperature and decreasing oxygen on coastal copepods // *Biol. Bull.* Vol.243. No.2. P.85–103. <https://doi.org/10.1086/722111>
- Roncalli V., Cieslak M.C., Castelfranco A.M., Hopcroft R., Hartline D.K., Lenz P.H. 2021. Post-diapause transcriptomic restarts: insight from a high-latitude copepod // *BMC Genomics.* Vol.22. Art.409. <https://doi.org/10.1186/s12864-021-07557-7>
- Rosowski J.R., Belk D., Gouthro M.A., Lee K.W. 1997. Ultrastructure of the cyst shell and underlying membranes of the brine shrimp *Artemia franciscana* Kellogg (Anostraca) during postencystic development, emergence, and hatching // *J. Shellfish Res.* Vol.16. P.233–249.
- Sanoamuang L., Dabseepai P. 2021. Diversity, distribution, and habitat occurrence of the diaptomid copepods (Crustacea: Copepoda: Diaptomidae) in freshwater ecosystems of Thailand // *Water.* Vol.13. Art.2381. <https://doi.org/10.3390/w13172381>
- Sichlau M.H., Hansen J.L.S., Andersen T.J., Hansen B.W. 2011. Distribution and mortality of diapause eggs from calanoid copepods in relation to sedimentation regimes // *Mar. Biol.* Vol.158. No.3. P.665–676.
- Tordesillas D.T., Abaya N.K.P., Dayo M.A.S., Marquez L.E.B., Papa R.D.S., Ban S. 2016. Effect of temperature on life history traits of the invasive calanoid copepod *Arctodiaptomus dorsalis* (Marsh, 1907) from Lake Taal, Philippines // *Plankton Benthos Res.* Vol.11. P.105–111.
- Watiroyram S., Sanoamuang L. 2017. A new species of *Mongolodiaptomus* Kiefer, 1938 from northeast Thailand and a key to the species (Crustacea, Copepoda, Calanoida, Diaptomidae) // *ZooKeys.* Vol.710. P.15–32. <https://doi.org/10.3897/zookeys.710.13941>
- Watras C.J. 1983. Reproductive cycles in diaptomid copepods: Effects of temperature, photocycle, and species on reproductive potential // *Can. J. Fish Aquat. Sci.* Vol.40. P.1607–1613.
- Williamson C.E., Reid J.W. 2001. Copepoda // J.H. Thorp, A.P. Covich (eds.). *Ecology and classification of North American freshwater invertebrates.* New York: Academic Press. P.915–954.
- Yoshida T., Liong C., Majid A.M., Toda T., Othman B.H.R. 2012. Temperature effects on the egg development time and hatching success of three *Acartia* species (Copepoda: Calanoida) from the Strait of Malacca // *Zool. Stud.* Vol.51. P.644–654.
- Zeller M., Jiménez-Melero R., Santer B. 2004. Diapause in the calanoid freshwater copepod *Eudiaptomus graciloides* // *J. Plankton Res.* Vol.26. No.12. P.1379–1388. <https://doi.org/10.1093/plankt/fbh128>